

Error-prone PCR

Subclone mutated inserts into YEphPR-B
Obtain region-specific random mutant libraries

Transform BJ3505/YRpPC3GS+
(trp- ura-)



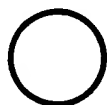
CAA/Cu plate

Replica plates
(CAA/Cu/XGal)

UP-MUTANTS

plates

phenotype
(blue)

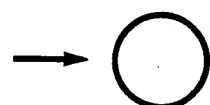


no hormone

Hormone-independent activation
of transcription



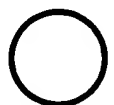
1uM Prog.



10nM Prog.

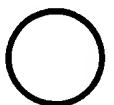
(or R5020)

Increased binding
affinity



1uM RU486

Altered
activity



1uM Dex

Altered
specificity

DOWN-MUTANTS

plates

phenotype
(white)



1uM Prog.

Inactivation
of
transcription

Fig.1

APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
DRAFTSMAN		

B

DNA sequence:

2636

WT ..AAC TTG CAT GAT CTT GTC AAA CAA CTT CAT CTG TAC TGC TTG...

UP-1 ..AAT TGC ATG ATC TTG TCA AAC AAC TTC ATC TGT ACT GCT IGA

Protein sequence:

879	WT	..Asn	Leu	His	Asp	Leu	Val	Lys	Gln	Leu	His	Leu	Tyr	Cys	Leu..
								★							
	UP-1	..Asn	Cys	Met	Ile	Leu	Ser	Asn	Asn	Phe	Ile	Cys	Thr	Ala	

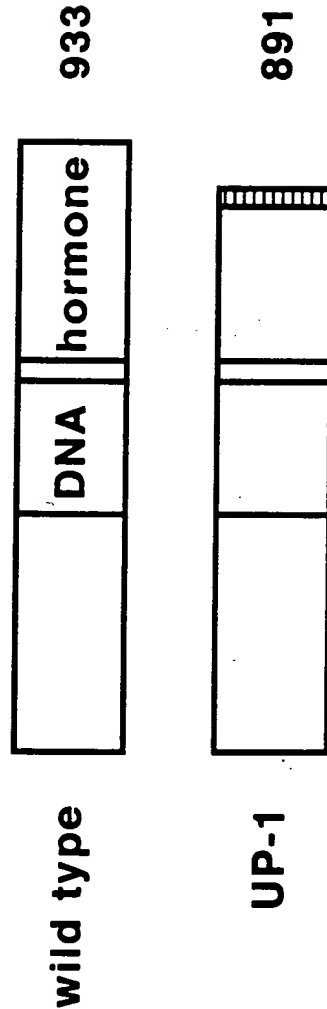


Fig.2B

003121-EEFSTUD

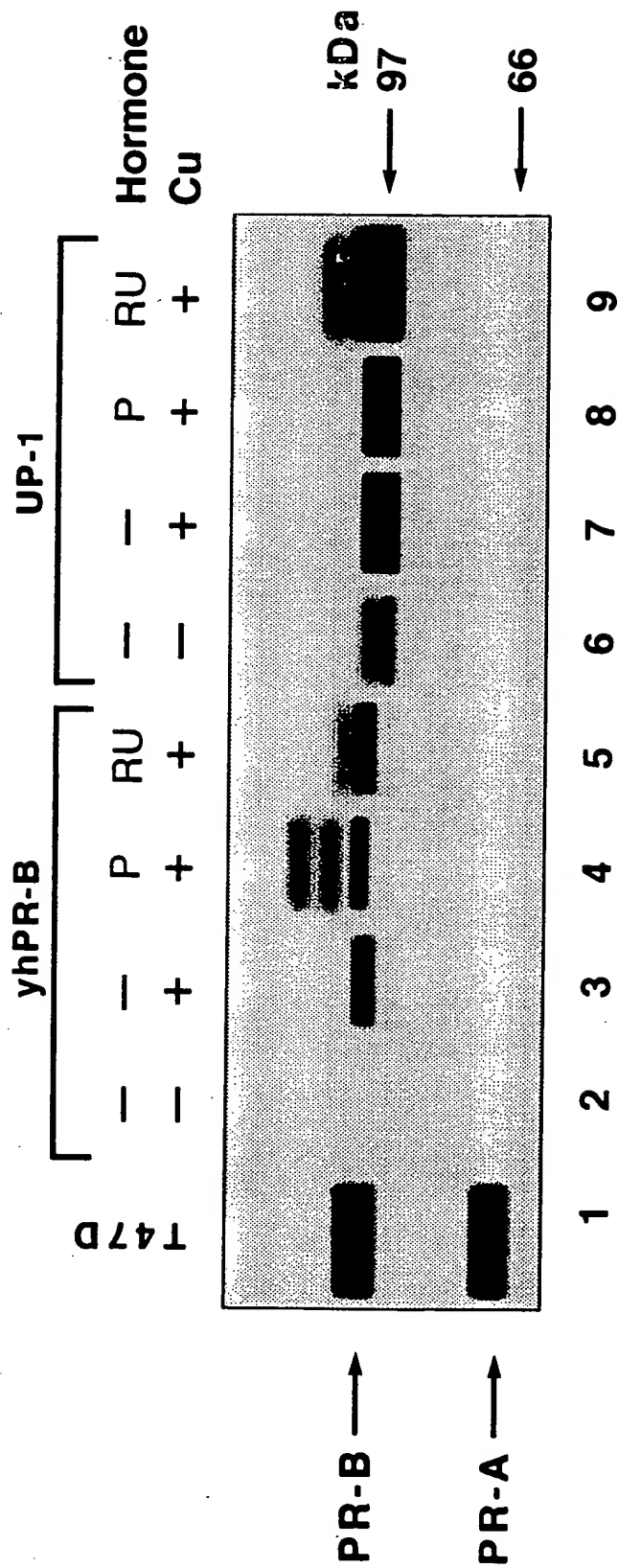


Fig.3

APPROVED	D.G. FIG.	
BY	CLASS	SUBCLASS
DRAFTSMAN		

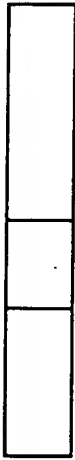



hPR Constructs	Transcriptional Activity (Miller Units)			Specific Binding (nM)		
	—	P	RU	P	P	RU
YEphPR-B (933)		86	6200	586	1.0	1.3
UP-1		286	466	8050	0.02	1.6
YEphPR-B879		166	242	5900	0.04	1.8
YEphPR-B891		243	226	6175	0.03	1.6

Fig.4

APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
DRAFTSMAN		

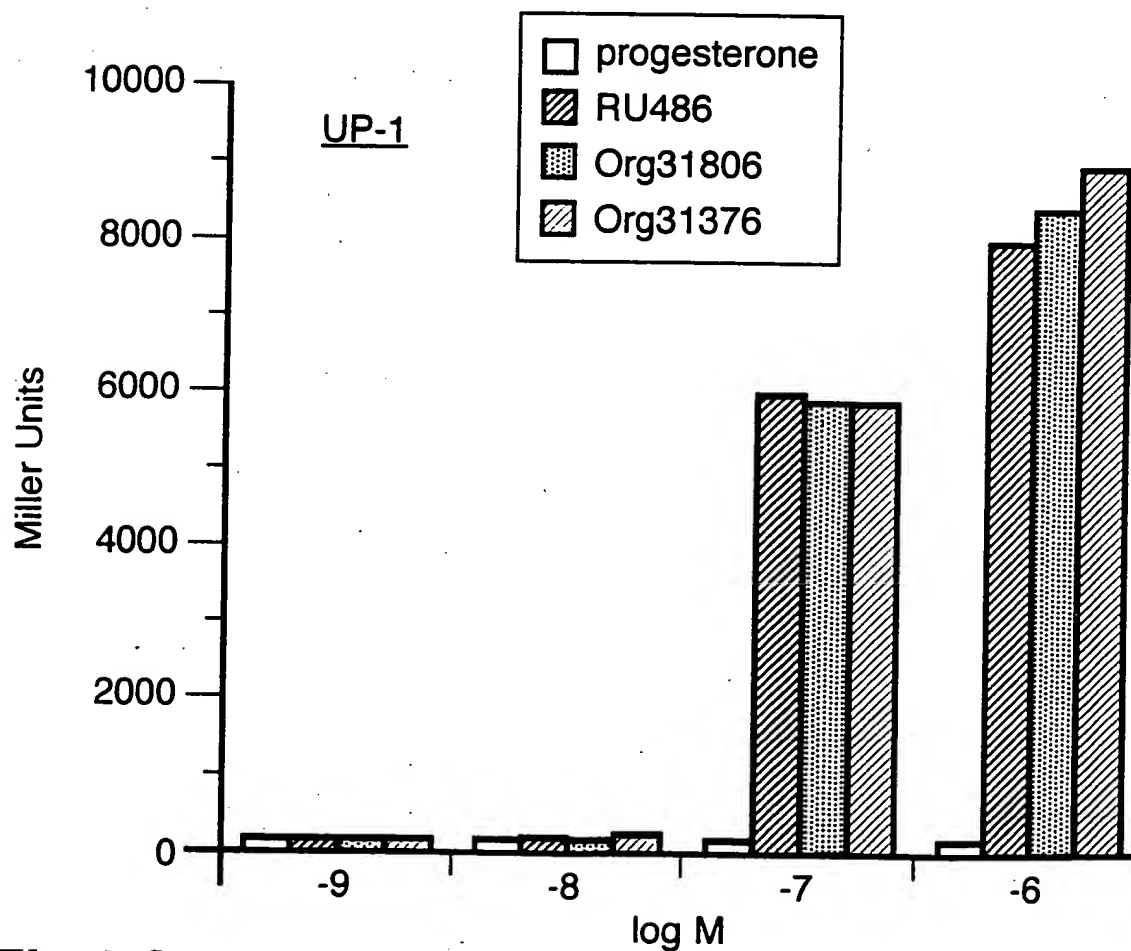
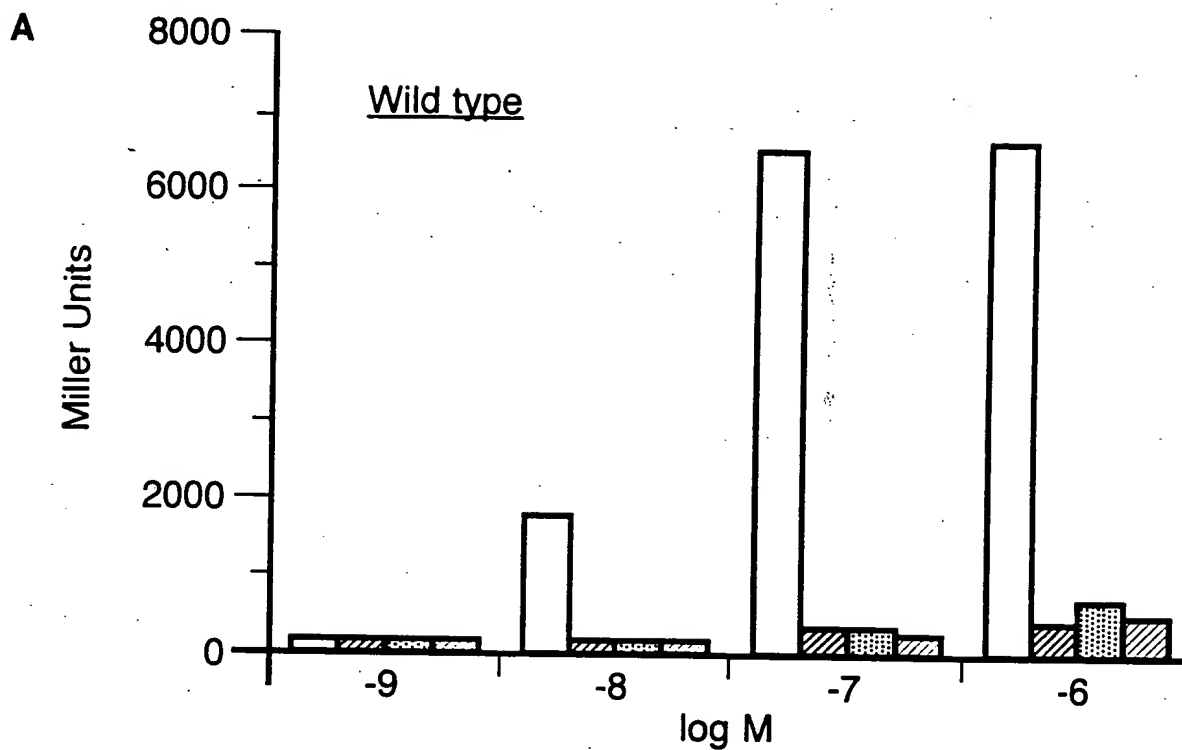


Fig.5A

APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
DRAFTSMAN		

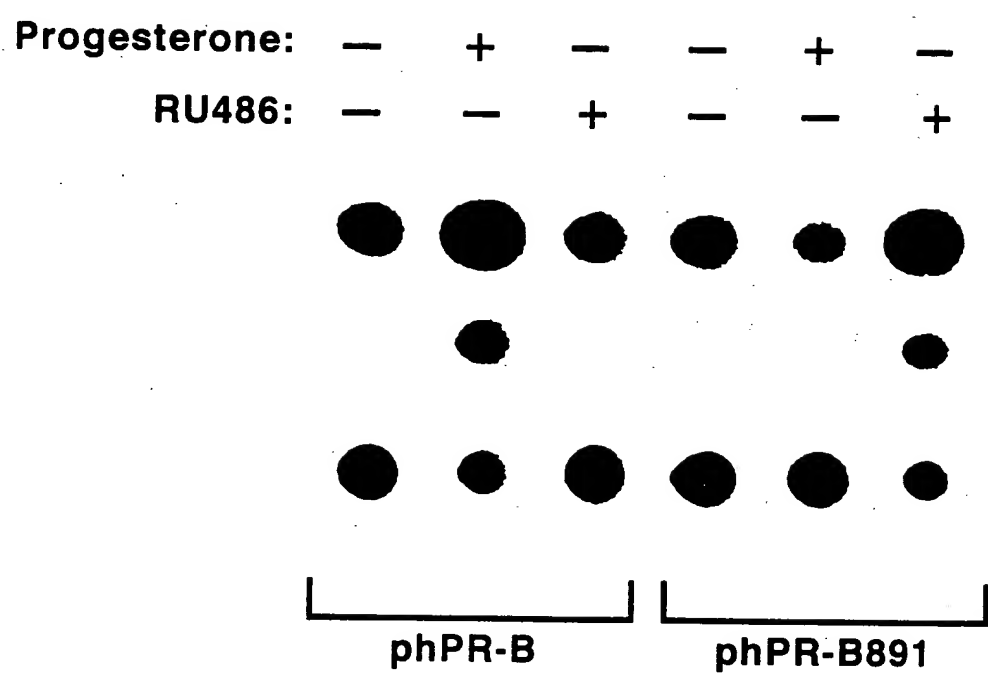


Fig.6A

APPROVED:	C.G. FIG.	
BY	CLASS	SUBCLASS
DRAFTSMAN		

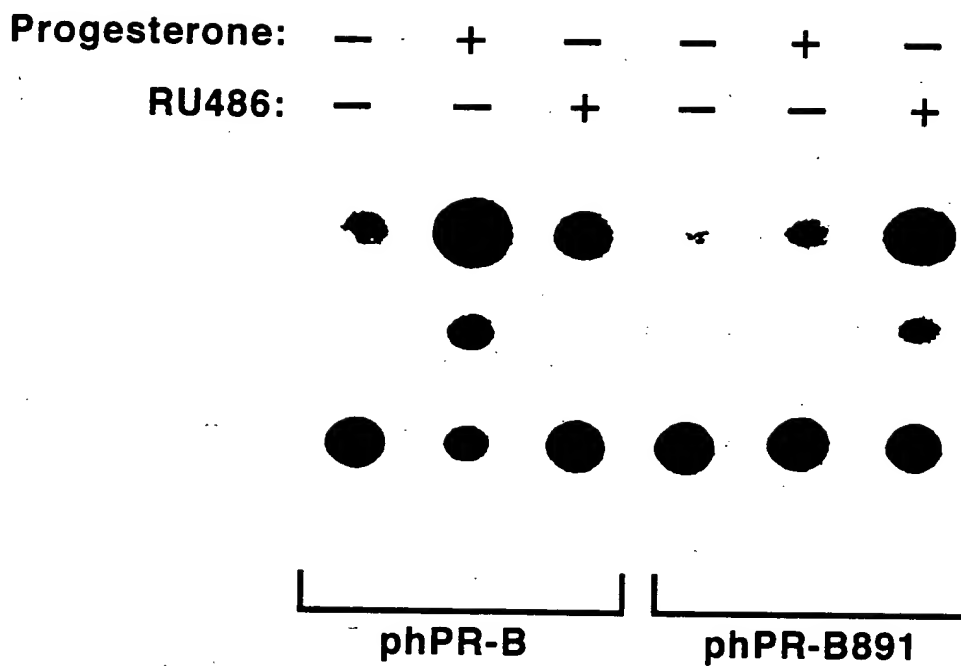


Fig.6B